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ORIGINAL PAPER

Effects of diet quality on phenotypic flexibility of organ size and digestive function in Mongolian gerbils (*Meriones unguiculatus*)

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Abstract In the context of evolution and ecology, there is a trade-off between the benefits of processing food through a digestive system with specific phenotypic attributes and the cost of maintaining and carrying the digestive system. In this study, we tested the hypothesis that digestive modulations at several levels can match each other to meet the energy and nutrient demands of Mongolian gerbils, a small granivorous rodent species, by acclimating them to a high-quality diet diluted with alfalfa powder. Mongolian gerbils on the diluted diet maintained metabolizable energy intake by an integrated processing response (IPR), which included increases in dry matter intake, gut capacity and rate of digesta passage after 2-weeks of acclimation. Down-regulation of hydrolytic enzyme activity in the intestinal brush-border membrane supported the adaptive modulation hypothesis. The absence of up-modulation of summed enzyme hydrolytic capacity on the diluted diet indicated that greater mass of small intestine on a high-fibre diet is not a direct indicator of digestive or absorptive capacity. Changes in mass of vital organs and carcass suggested that the amount of energy allocated to various organs and hence physiological functions was regulated in response to diet shift.

Keywords Adaptive modulation hypotheses · Integrated processing response (IPR) · Mongolian gerbils · Gastrointestinal tract · Sucrase · Maltase · Aminopeptidase-*N* · Metabolizable energy intake · Vital organs

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Introduction

In the context of evolution and ecology, there is a trade-off between the benefits of processing food through a digestive system with specific phenotypic attributes (e.g. gut mass and length, the density and activity of intestinal enzyme and nutrient transporters) and the cost of maintaining and carrying the digestive system (Sibly 1981; Diamond and Karasov 1983; Toloza et al. 1991; Diamond 1991; Derting and Bogue 1993; Naya et al. 2005). For example, the digestive and absorptive capacity of the vertebrate small intestine should be matched to nutrient and energy intake through natural selection (Diamond 1991; Karasov and Hume 1997). If there were no such match, then valuable food energy might be wasted in excreta when feeding on diets with high substrate levels, and/or the metabolic expenses of synthesizing and maintaining the molecular machinery to hydrolyze and absorb substrate would be wasted when feeding on diets with very

low levels of substrate (Diamond 1991; Karasov and Hume 1997). Based on this premise of economical design, the adaptive modulation hypothesis (Karasov and Diamond 1983; Ferraris and Diamond 1989) had been proposed to explain the match between digestive enzymes and their substrates.

Adaptive modulations in gut morphological and physiological traits during a diet shift are important for an animal to maintain energy balance (Karasov 1996; Piersma and Lindström 1997; Starck 1999a, b). Changes in gut morphology (e.g. Gross et al. 1985; Green and Millar 1987; Hammond and Wunder 1991; Starck 1999a, b; Pei et al. 2001a, b; Lentle et al. 2004; Munn et al. 2006), retention time of food in the gut and food distribution in digestive regions (e.g. Sakaguchi et al. 1987; Dykstra and Karasov 1992; Hume et al. 1993; Pei et al. 2001a, b), hydrolytic enzyme activity (e.g. Martinez del Rio et al. 1995; Sabat et al. 1998; Sabat and Bozinovic 2000), and nutrient transport density and activity (Karasov et al. 1983; Toloza et al. 1991; Buddington et al. 1991; Caviedes-Vidal and Karasov 1996; Afik et al. 1997) in the brush-border membrane of the intestine with a shift in diet have been reported in many vertebrates. All these studies show that digestive phenotypic flexibility can appear at different organizational levels, ranging from organs to molecules.

In herbivorous animals, the relationships between gut morphology, functional capacity (e.g. enzyme activity), food intake and retention can all change in response to changes in food quality. The effect of these changes on digestible energy or nutrient intake was named the integrated processing response (IPR) hypothesis (Batzli et al. 1994; Young Owl and Batzli 1998). The IPR can maintain many herbivorous animals' required intake of digestible dry matter or energy on diets with higher fibre content, because of the increase in food intake, gastrointestinal (GI) tract size, absorptive capacity (epithelial mass) of the GI tract, and decrease in digesta mean retention time. Several studies on voles support the IPR hypothesis (Gross et al. 1985; Hammond and Wunder 1991; Batzli et al. 1994; Young Owl and Batzli 1998; Pei et al. 2001a). However, these studies did not measure hydrolytic enzyme activity, nutrient transport density, or activity in the brush-border membrane of the intestine during a diet shift. The greater mass of small intestine in animals fed low-quality (higher fibre) food was attributed to a greater epithelial mass, and hence the improvement of digestive or absorptive capacity of the intestine. This opinion is contrary to the prediction by the adaptive modulation hypothesis which predicts that low food quality will down-regulate digestive enzyme and nutrient transport specific-mass activity.

In the present study, we tested whether digestive modulations at several organizational levels match each other; thereby meeting energy and nutrient demands. We used a small granivorous rodent, the Mongolian gerbil (*Meriones unguiculatus*), acclimated to high-quality food diluted with alfalfa powder. Alfalfa powder was used because it has a high fibre content, which cannot be hydrolysed by endogenous digestive enzymes of vertebrates; Mongolian gerbils feed mainly on low-fibre green stems and leaves in summer (Xia and Zhong 1966). We predicted that Mongolian gerbils could maintain their metabolizable energy intake by an IPR, including increase in dry matter intake, gut size and rate of digesta passage when fed the diluted diet. Based on the adaptive modulation hypothesis, however, we predicted that the hydrolytic enzyme activity in the brush-border membrane of the intestine would be down-regulated in gerbils fed the diluted diet. We also were interested in determining gross phenotypic changes in other vital organs such as heart, lungs, liver, spleen, kidneys and testis as energy demand and diet quality varied, since masses of the organs can be used as an index of basal energy requirements (Wunder 1992; Speakman and McQueenie 1996; Hammond and Janes 1998; Hammond et al. 1999; Derting and Hornung 2003).

Materials and Methods

Animals and diets

All animal procedures were approved by the Institutional Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Adult male gerbils were from our laboratory colony, which was derived from gerbils live-trapped in Inner Mongolia grassland in 1999. They were housed alone in individual cages (30 cm × 20 cm × 15 cm) with sawdust bedding under a constant light cycle (16:8 h light-dark cycle) and temperature (22 ± 1°C), and maintained on a commercial standard rat pellet (Beijing Ke Ao Feed Co.). Twelve adult male gerbils were divided randomly into two groups, and acclimated to one of two diets (Table 1) for 14 days. Zhao (2006) found that intake of the diluted diet stabilised within this period. Food and water were available ad libitum. The diluted diet was prepared by thoroughly mixing three parts of alfalfa powder to one part (by dry mass) of powdered commercial standard rat pellets of the same particle size (Beijing Ke Ao Feed Co). The dry mixture was moistened and re-pelleted (12 mm diameter) (K-L-S, Shanghai Jia Le Feed Machine Co.) and then dried outdoors.

Table 1 Nutrient composition (% dry mass) and gross energy content (kJ/g dry mass) of two diets fed to Mongolian gerbils

Nutritional parameter	Standard diet	Diluted diet
Crude fat	6.1 ± 0.8	4.8 ± 0.9
Crude protein	34.3 ± 3.5	21.7 ± 1.5
Neutral detergent fiber (NDF)	13.5 ± 1.3	37.6 ± 2.5
Acid detergent fiber (ADF)	6.5 ± 0.5	26.3 ± 3.3
Gross energy (kJ/g)	17.69 ± 0.01	17.84 ± 0.01

Note. Values are means ± SE (n = 5)

Food intake and digestibility

After an acclimation period of 14 days on the experimental diets, food residues and faeces were collected from each animal for 24 h. Zhang (2005) measured food intake daily for 7 days in diet-acclimated Mongolian gerbils and found a coefficient of variation of only 3.4% around the mean intake value. Thus a 1-day collection period was deemed appropriate for this small rodent. Similarly short collection periods have been used for other small rodents by other researchers (e.g. Toloza et al. 1991; Johnson et al. 2001; Derting and Compton 2003; Król and Speakman 2003). The animals were weighed (±0.1 g) at the beginning and end of the food intake trial. All food residues, faeces and samples of the food were oven-dried for 5 days at 60°C and separated manually, and then their dry masses recorded (±1.0 mg). The gross energy contents of food and faeces were determined in a Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA) with benzoic acid as the standard.

Gastrointestinal tract morphology

All animals were sacrificed between 0900 and 1000 hours by puncture of the posterior vena cava after the measurement of food intake. Immediately afterwards, the abdominal cavity was opened and the entire gastrointestinal tracts were removed and dissected free of mesenteric attachments on a square glass with an ice-containing tray below. The length of stomach, small intestine (SI), caecum and colon were measured by extending the organ to its unstressed length along a ruler (±1.0 mm, Hammond and Wunder 1991; Pei et al. 2001a, b). After recording the wet mass (±1.0 mg) with contents, these organs were opened and the contents removed, and then each organ was rinsed in cold Ringer's solution, blotted dry on tissue paper, and weighed. For each gerbil, three 0.8–1.0 cm pieces of small intestines were weighed, placed in an Eppendorf tube, and stored in liquid nitrogen for enzyme assays. The three pieces of tissue came from the duodenum (proximal SI), jejunum (mid-region SI) and ileum (distal SI). The wet carcass was weighed (±1.0 mg) after removal of vital organs and gut with contents. The content-free gut tissues were dried at 60°C for 7 days and weighed (±1.0 mg). The mass of contents was assumed to be the difference between the wet mass of a segment with and without contents. The total dry mass of the SI was estimated by multiplying the total wet mass of the SI by the ratio between the dry mass to the wet mass of residual SI tissue.

Vital organ mass

The heart, lungs, liver, kidneys, spleen and both testis were removed, cleared of fat and connective tissue, blotted dry and weighed (±1.0 mg). Masses were measured again after drying each organ to a constant mass in an oven at 60°C.

Enzyme activity assays

We examined the effect of diet dilution on the activity of three digestive enzymes in the intestinal brush-border membrane: sucrase (E.C. 3.2.1.48), maltase (E.C. 3.2.1.20) and aminopeptidase-*N* (E.C. 3.4.11.2). We chose the disaccharidases as indicators of a gerbil's ability to assimilate polysaccharides such as starch and amylopectin, and the dipeptidase as an indicator of protein hydrolysis, which can account for almost all of the peptidase activity of the brush border membrane (Maroux et al. 1973). Assays were performed in duplicate, with a mean coefficient of variation of less than 0.5%.

Sample preparation

Intestinal tissue samples were thawed at 4°C and homogenized (30 s, using a homogenizer maximum setting) in 0.9% NaCl (1:10, w/v) in an ice-water bath. We measured activity of membrane-bound enzymes in whole tissue homogenates rather than in mucosal samples or isolated brush border membrane preparations to avoid underestimation of activity as reported previously (Martínez del Río 1990).

Dissacharidase activity assays

We determined the activity of sucrase and maltase in the SI homogenate using the colorimetric method developed by Dahlqvist (1984) and modified by Martínez del Río (1990). In brief, 100 µl of appropriately diluted tissue homogenate was incubated with 100 µl of 56 mM sugar (sucrose and maltose) solutions in 0.1 M maleate/NaOH pH 6.5. After 10 min of incubation at 37°C, we arrested the reaction by adding 3 ml of Glucose kit

(Beijing BHKT Clinical Reagent Co., Ltd, Beijing, China). The sample solution was allowed to stand for 20 min and then the absorbance measured at 505 nm with a Beckman DU-800 spectrophotometer. Enzyme activity was determined using a glucose standard curve.

Aminopeptidase-N assay

Aminopeptidase-*N* assays were carried out using L-alanine-*p*-nitroanilide as a substrate (Maroux et al 1973). We started the reaction by mixing 10-µl tissue homogenate with 1 ml assay solution, made of 2.04 mM L-alanine-*p*-nitroanilide in 0.2 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.0). The reaction was initiated by incubation at 37°C and then arrested after 10 min with 3 ml chilled 2 N acetic acid. The absorbance was measured at 384 nm, and activity was determined using a *p*-nitroanilide standard curve.

The protein content of SI tissue was measured with Folin phenol reagent (Sigma) with bovine serum albumin as the standard. Absorbance was read at 500 nm (Assay type: Lowry-Low Resolution). We calculated summed and standardized intestinal activities for all enzymes. Data on enzyme activities are presented as total hydrolytic activity (µmol min^{-1}) and activity per g of protein (µmol min^{-1} g $protein^{-1}$). The advantages of our normalization procedures are discussed by Martínez del Río et al. (1995). We calculated the summed hydrolytic activity of the entire small intestine by multiplying the average tissue-specific activity (µmol min^{-1} g wet $tissue^{-1}$) in all three regions by the SI total wet mass.

Calculations and data analysis

Digestive parameters were calculated as follows:

Dry matter intake (g/day) = total dry matter (g/day) − dry mass of food residues (g/day);
Digestible dry matter intake (g/day) = dry matter intake (g/day) - dry mass of faeces (g/day);
Apparent dry matter digestibility (%) = digestible dry matter intake (g/day) × 100/dry matter intake (g/day);
Gross energy intake (kJ/day) = dry matter intake (g/day) × gross energy content of food (kJ/g);
Faeces gross energy (kJ/day) = dry mass of faeces (g/day) × gross energy content of faeces (kJ/g);
Digestible energy intake (kJ/day) = gross energy intake (kJ/day) − faeces gross energy (kJ/day);
Apparent energy digestibility (%) = digestible energy intake (kJ/day) × 100/gross energy intake (kJ/day);
Turnover time (h) = total mass of gut contents (g) × 24 (h)/daily dry matter intake (g) (Penry and Jumars 1987; Hammond and Wunder 1991).
Turnover time per g digesta (h/g) = turnover time (h)/total mass content of gut (g).

All results are shown as means ± 1SE. Prior to all statistical analyses, data were tested for normality and homogeneity of variance using Kolmogorov–Smirnov and Levene tests, respectively. Differences between two groups were tested by independent-sample *t* tests. To examine the effects of food quality and intestinal region on digesta distribution, turnover time, SI tissue protein content, and enzyme activities, we used repeated measures analysis of variance (RM-ANOVA). All statistical tests were performed with SPSS. The significance level was set at $P < 0.05$ and $0.05 < P < 0.10$ was taken to indicate a trend.

Results

Body mass, food intake and digestibility

There were no differences in body mass between the two groups of gerbils either at the beginning or at the end of the acclimation period (Table 2), or within each group during the 2-week acclimation period ($P > 0.10$, Paired *t* test). However, the wet carcass mass was 17% lower ($P < 0.05$) in gerbils eating the diluted diet, which indicated that these gerbils lost mass (Table 2).

Gerbils consuming the diluted diet had 82% higher food intake than did gerbils fed on the standard diet. However, faecal output was more than threefold greater in gerbils fed on the diluted diet (Table 2). Digestible energy intake was similar on both diets. Animals on the diluted diet had almost sixfold greater faecal energy loss, but had a near double gross energy intake than animals on the standard diet (Table 2). The apparent digestibility of dry matter and energy was lower in gerbils fed on the diluted diet than in gerbils fed on the standard diet, by 43 and 47%, respectively.

Organ size changes

Vital organ mass

The wet masses of the liver and kidneys were greater in gerbils fed on the diluted diet (by 25 and 12%, respectively) compared with gerbils on the standard diet. The wet mass of the spleen was 28% less on the diluted diet. There were no significant differences in the wet masses of the lungs or testis between the two groups.

Table 2 Food intake and digestive efficiency of Mongolian gerbils fed two experimental diets

	Standard diet $n = 6$	Diluted diet $n = 6$	t	P
Body mass (g)				
Before acclimated	60.8 ± 1.6	58.8 ± 3.5	0.518	0.616
Initial	62.0 ± 1.5	59.8 ± 3.7	0.555	0.591
Final	60.8 ± 1.5	60.5 ± 3.5	0.078	0.939
Carcass[a]	47.5 ± 1.8	39.4 ± 2.9	2.372	0.039
Dry Matter (g/day)				
Intake	6.34 ± 0.23	11.52 ± 0.58	−8.257	<0.001
Fecal output	1.57 ± 0.12	6.62 ± 0.35	−13.615	<0.001
Energy (kJ/day)				
Gross intake	112.14 ± 4.07	205.60 ± 10.42	−8.352	<0.001
Fecal output	20.39 ± 1.48	116.71 ± 6.02	−15.541	<0.001
Digestible intake	91.75 ± 3.10	88.89 ± 5.18	0.475	0.645
Apparent digestibility (%)				
Dry matter	75.28 ± 1.37	42.55 ± 1.22	17.868	<0.001
Energy	81.89 ± 0.96	43.20 ± 1.09	26.658	<0.001
Turnover time (h)				
Total digesta	11.1 ± 1.2	18.1 ± 1.3	−4.044	0.002
per g of digesta	3.81 ± 0.14	2.11 ± 0.10	9.877	<0.001

Note. Values were means ± SE

[a] Carcass mass: body mass without vital organs and gut with contents

The dry mass of the heart on the diluted diet was 17% lighter than on the standard diet, although there was a non-significant trend ($t = 2.166$; $P = 0.056$) for lower heart wet mass (15%) on the diluted diet. The dry mass of the liver was 19% higher on the diluted diet relative to the standard diet (Table 3).

Gut size

Gerbils fed the diluted diet had 18% longer and 47% greater wet mass of the GI tract than animals fed the standard diet, and total dry mass of the GI tract tended to be greater (23%, $t = -2.085$, $P = 0.064$; Table 3). The length of the small intestine (SI), caecum and colon increased 14, 43 and 17%, respectively, in response to the diluted diet. The length of the stomach was similar between groups. In gerbils maintained on diluted diet, the wet masses of the stomach, caecum and colon were significantly greater by 34, 107 and 91%, respectively, than on the standard diet. The wet mass of the SI tended to be heavier (by 25%, $t = -2.137$, $P = 0.071$). The dry masses of the caecum and colon were 67 and 59% greater than those of gerbils on the standard diet. For the SI, there was no difference in dry mass between groups (Table 3).

Gut contents

Gerbils maintained on the diluted diet showed increase in the wet contents mass of the total gut (194%), stomach (59%), SI (224%), caecum (281%) and colon (196%) relative to gerbils fed the standard diet (Table 3). Diet had a significant effect on digesta distribution in the digestive regions ($F_{3,30} = 10.855$, $P < 0.001$, RM-ANOVA); gerbils on the diluted diet had a lower proportion of digesta in the stomach and higher proportion in the caecum than those on the standard diet (Fig. 1). The proportions of digesta in the small intestine and colon were not affected by diet dilution (Fig. 1). Total turnover times on the diluted diet were greater than those on the standard diet (Table 2), however, average per g digesta turnover time was 45% shorter in gerbils fed the diluted diet (Table 2).

Activity of digestive enzymes

Specific activity

Diet had a significant effect on the protein concentration of SI tissue ($F_{1,10} = 10.556$, $P = 0.009$, RM-ANOVA): gerbils fed the diluted diet had, on average, 13% higher protein content than those fed the standard diet. Protein concentration varied with position in the SI, with the lowest concentration in the ileum (Fig. 2). Therefore, we standardized our enzyme activity results per g protein.

Consistent with our prediction, the diluted diet was associated with a negative effect on sucrase ($F_{1,10} = 5.457$, $P = 0.042$) and aminopeptidase-N ($F_{1,10} = 18.976$, $P = 0.001$), and a trend for maltase ($F_{1,10} = 3.716$, $P = 0.083$) activity. In the ileum, activities of all three enzymes were lower in gerbils fed on the diluted diet than that in gerbils on the standard diet (Fig. 2).

Table 3 Organ sizes in Mongolian gerbils fed two experimental diets (mean ± SE)

	Standard diet $n = 6$	Diluted diet $n = 6$	t	P
Organ wet mass (g)				
Heart	0.269 ± 0.015	0.229 ± 0.010	2.166	0.056
Lungs	0.382 ± 0.033	0.366 ± 0.024	0.404	0.695
Liver	1.848 ± 0.057	2.310 ± 0.135	−3.151	0.010
Testis	0.713 ± 0.073	0.733 ± 0.039	−0.240	0.815
Spleen	0.036 ± 0.002	0.026 ± 0.004	2.319	0.043
Kidneys	0.591 ± 0.019	0.664 ± 0.024	−2.385	0.038
Organ dry mass (g)				
Heart	0.063 ± 0.004	0.052 ± 0.002	2.640	0.025
Lungs	0.083 ± 0.007	0.076 ± 0.005	0.737	0.478
Liver	0.576 ± 0.024	0.684 ± 0.041	−2.248	0.048
Testis	0.118 ± 0.012	0.110 ± 0.006	0.557	0.589
Spleen	0.007 ± 0.001	0.006 ± 0.001	0.850	0.415
Kidneys	0.143 ± 0.005	0.153 ± 0.007	−1.253	0.239
Length of gut segment (cm)				
Stomach	3.2 ± 0.1	3.6 ± 0.2	−1.471	0.172
Small intestine	31.2 ± 0.6	35.7 ± 0.6	−5.093	<0.001
Caecum	5.1 ± 0.2	7.3 ± 0.1	−9.053	<0.001
Colon	14.1 ± 0.4	16.5 ± 0.3	−5.064	<0.001
Wet mass of gut segment (g)				
Stomach	0.476 ± 0.021	0.636 ± 0.051	−2.905	0.016
Small intestine	1.460 ± 0.067	1.824 ± 0.157	−2.137	0.071
Caecum	0.340 ± 0.022	0.704 ± 0.073	−4.753	0.003
Colon	0.393 ± 0.018	0.749 ± 0.061	−5.588	<0.001
Dry mass of gut segment (g)				
Stomach	0.106 ± 0.005	0.127 ± 0.010	−1.890	0.088
Small intestine	0.286 ± 0.013	0.301 ± 0.031	−0.422	0.682
Caecum	0.060 ± 0.004	0.100 ± 0.009	−4.059	0.002
Colon	0.082 ± 0.003	0.130 ± 0.009	−5.217	<0.001
Contents of gut segment (g)				
Stomach	0.821 ± 0.092	1.307 ± 0.183	−2.374	0.039
Small intestine	0.617 ± 0.055	1.999 ± 0.196	−6.805	<0.001
Caecum	1.050 ± 0.146	4.003 ± 0.332	−8.151	<0.001
Colon	0.450 ± 0.093	1.333 ± 0.129	−5.533	<0.001
Totals for entire gut				
Length	53.6 ± 0.4	63.1 ± 1.0	−9.027	<0.001
Wet mass	2.669 ± 0.102	3.914 ± 0.315	−3.762	0.009
Dry mass	0.534 ± 0.022	0.657 ± 0.055	−2.085	0.064
Contents	2.937 ± 0.319	8.642 ± 0.561	−8.844	<0.001

Note Values are means ± SE

Moreover, down-regulations in enzyme activity occurred in the duodenum for aminopeptidase-*N* and in jejunum for sucrase (Fig. 2). The differences between groups ranged from 23% for sucrase in the jejunum to 43% for aminopeptidase-*N* in the ileum. The activities of all three enzymes (sucrase, maltase and aminopeptidase-*N*) were greater in the jejunum than in either the duodenum or ileum, regardless of diet (Fig. 2).

Summed enzyme capacity

There were no significant differences between the two diet treatments in the summed hydrolysis rates for sucrase, maltase and aminopeptidase-*N* (Fig. 3).

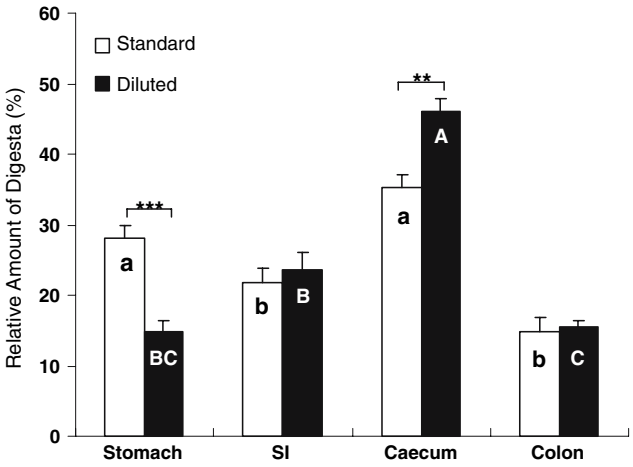

Fig. 1 Relative amount of digesta in each digestive organ in Mongolian gerbils fed standard diet (*white panes*) and diluted diet (*black panes*). *Bars* are means ± SE ($n = 6$). Bars that share a common letter (*lowercases* standard diet; *capitals*, diluted diet) reflect means that are not significantly different (RM-ANOVA). Differences between two treatments were tested by t tests, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Relationship between sucrase, maltase and aminopeptidase-N

Intestinal sucrase and maltase activities were positively correlated in the duodenum ($r = 0.874$, $P < 0.001$), jejunum ($r = 0.883$, $P < 0.001$) and ileum ($r = 0.906$, $P < 0.001$) (Fig. 4a). Sucrase activity was also positively correlated with aminopeptidase-N in the jejunum ($r = 0.626$, $P = 0.029$) and ileum ($r = 0.789$, $P = 0.002$), but not the duodenum ($r = 0.292$, $P = 0.357$) (Fig. 4b).

Discussion

An entire process is optimal only when each stage functions optimally with respect to the preceding stage and successive (Penry and Jumars 1987). Thus there is a need to match the rate of feeding with the rates of digesta transit, digestion and absorption in order to maximize metabolizable energy and nutrient intake and minimize costs of time and energy. This match pattern should vary with changes in food quality and energy demand. In our study, Mongolian gerbils fed on a diluted diet maintained metabolizable energy intake by an IPR, including increase in dry matter intake, gut size and rate of digesta passage after 2 weeks of acclimation. The down-regulation of hydrolytic enzyme activity in the intestinal brush-border membrane was consistent with the prediction of the adaptive modulation hypothesis (Karasov and Diamond 1983; Ferraris

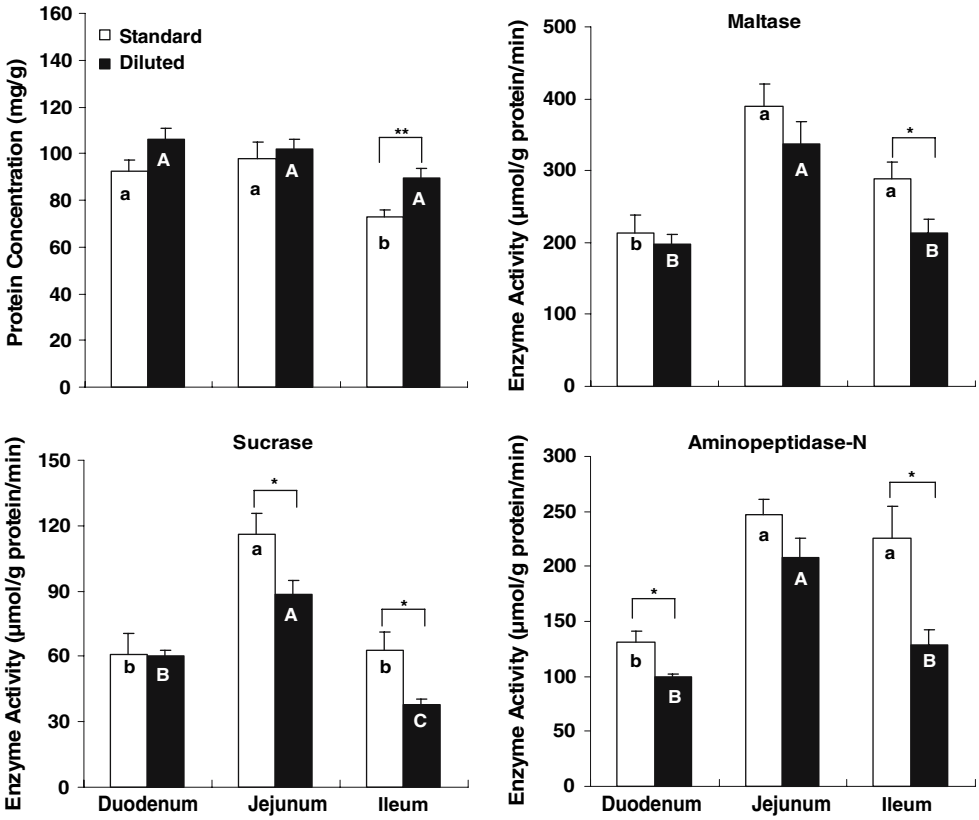

Fig. 2 Protein concentration of small intestinal tissue and digestive enzyme activity in Mongolian gerbils fed standard diet (*white panes*) and diluted diet (*black panes*). Bars are means ± SE ($n = 6$). Bars that share a common letter (*lower cases* standard diet; *capitals* diluted diet) reflect means that are not significantly different (RM-ANOVA). Differences between two treatments were tested by *t* tests, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

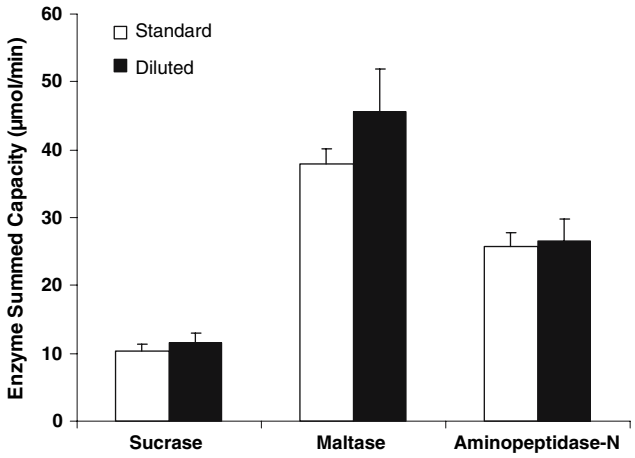

Fig. 3 Small intestinal summed hydrolysis capacity in Mongolian gerbils fed on standard (*open bars*) and diluted (*filled bars*) diets. *Bars* are means ± SE ($n = 6$)

and Diamond 1989). Changes in masses of vital organs suggested that the amount of energy allocated to various organs and hence physiological functions was regulated in response to diet shift.

Food intake and digestibility

In general, small rodents increase their food intake to meet energy and nutrient requirements with increasing fibre content in food (Kanarek et al. 1977; Gross et al. 1985; Hammond and Wunder 1991; Nagy and Negus 1993; Batzli et al. 1994; Bozinovic 1995; Castle and Wunder 1995; Young Owl and Batzli 1998). Although Mongolian gerbils did not significantly increase their food or energy intake when fibre content in food was added to a low level (15% nonnutritive celluflour, Kanarek et al. 1977; 19% ADF, Pei et al. 2001b), they almost doubled dry matter and/or energy intake on a higher fibre content diet (35–45% nonnutritive celluflour, Kanarek et al 1977; 26% ADF, this study). Bozinovic (1995) found that *Octodon degus* maintained on a 57% NDF diet had a 30–40% higher food intake than that of animals on 35 and 47% NDF diets; differences between groups maintained on 35 and 47% NDF diets were not significant. However, Castle and Wunder (1995) reported a good linear relationship between food intake and fibre content (NDF) of diets in *Microtus ochrogaster*, which were fed diets with fibre content from 20 to 84% by the means that animals received a new higher-fibre diet every 15 days. Their data suggested that the extent of food intake response to the varied fibre contents depend on the diet experience of animals as well as the fibre form and content.

By contrast, apparent digestibility of dry matter and energy were nearly halved in gerbils fed the diluted diet. A decline in digestibility has generally been found

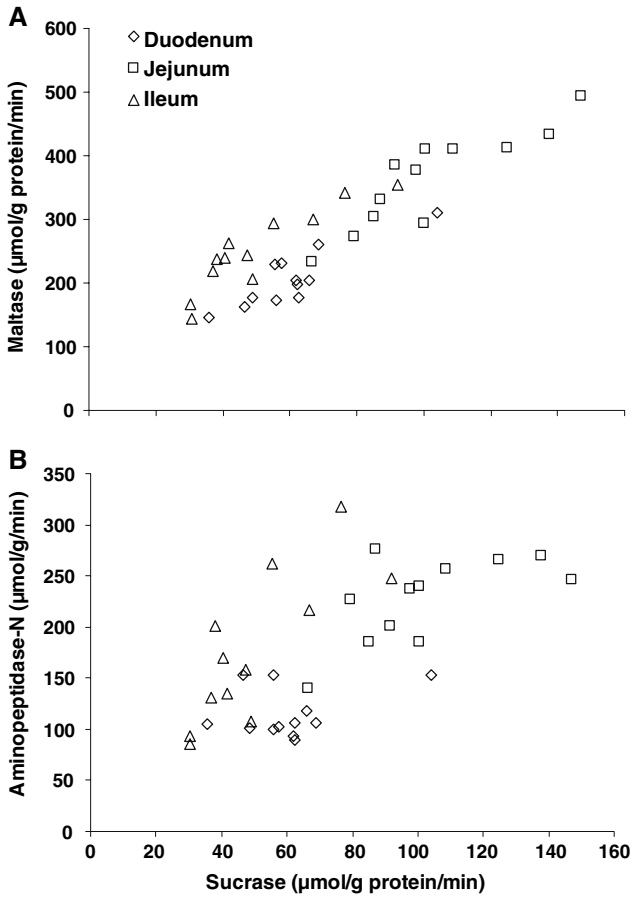

Fig. 4 Relationship between intestinal sucrase activity and maltase (**a**) and aminopeptidase-*N* activities (**b**) in Mongolian gerbils fed standard and diluted diets

in other small rodents on a high-fibre diet (Hammond and Wunder 1991; Nagy and Negus 1993; Batzli et al. 1994; Bozinovic 1995; Young Owl and Batzli 1998; Pei et al. 2001a, b). Nevertheless, the digestible dry matter and/or energy intake of gerbils fed the diluted diet was maintained, as occurs in most small herbivores consuming diets of a range of fibre contents (Hammond and Wunder 1991; Nagy and Negus 1993; Batzli et al. 1994; Castle and Wunder 1995; Young Owl and Batzli 1998; Pei et al. 2001a). The maintenance of digestible energy intakes may be attributed to an IPR, including increase in dry matter intake, gut size, rate of digesta passage and turnover time of digesta in response to a high-fibre diet.

Digestive organ size and digesta fill

The strategy of increasing gut size in response to a high-fibre diet is common in small herbivorous rodents (Gross et al. 1985; Green and Millar 1987; Hammond and Wunder 1991; Nagy and Negus 1993; Batzli et al. 1994; Young Owl and Batzli 1998; Pei et al. 2001a, b). The potential benefits from greater gut size include an increase in the turnover and retention time of digesta, food intake (Hammond and Wunder 1991; Batzli et al. 1994; Young Owl and Batzli 1998), and the amount of food processed by the gut in a given period (Gross et al. 1985; Green and Millar 1987; Hammond and Wunder 1991; Nagy and Negus 1993). In the present study, gerbils fed the diluted diet showed a near doubling in the total capacity of the gut when their food intake was 82% higher. Consequently, total digesta turnover time was delayed 7 h despite the higher food intake. Apparently, these changes contributed to maintaining digestible dry matter and energy intake.

Digestive enzyme activity

As expected, based on our prediction from the adaptive modulation hypothesis, protein-specific sucrase, maltase and aminopeptidase-*N* activities were down-regulated in Mongolian gerbils fed the diluted diet in response to the decreasing in substrates concentrations. These down-regulations occurred mainly in the ileum. The different responses of duodenal, jejunal and ileal enzyme activity to change in dietary substrates may be due to different luminal nutrient concentrations along the SI (Karasov and Hume 1997). Ileal enzyme activity showed the most dramatic modulation in our study, probably because duodenal enzyme activity is in a perpetually induced state due to a higher concentration of luminal nutrients (Karasov and Hume 1997).

The absence of any increase in summed or protein-specific enzyme activity indicated that the greater SI mass in gerbils fed the diluted diet was not a good indicator of epithelial mass or of SI digestive or absorptive capacity. The IPR of Batzli et al. (1994) and Young Owl and Batzli (1998) involves the response of epithelial mass and digestive or absorptive capacity, although no related parameters were measured directly. These authors based their conclusion on other studies (Karasov and Diamond 1988; Ferraris et al. 1989; Derting and Bogue 1993) that were not related to adaptation to high-fibre diets. In two other studies (Gross et al. 1985; Hammond and Wunder 1991) the effects of fibre content and cold ambient temperatures were not clearly distinguishable. In fact, the digestive and/or absorptive capacity of the SI could be un-specifically up-regulated as a result of greater mass of SI with increasing energy demands (e.g. Derting and Bogue 1993; Hammond and Diamond 1992; Hammond et al. 1994; Karasov and Hume 1997). On the diet diluted with fibre, the greater mass of the SI could be attributed mainly to an increase in thickness of small intestinal muscle layers related to expanded bulk and enhanced motility. For example, Starck and Rahmaan

(2003) reported that the muscle layer thickness of the SI increased 40–80% in quail fed a high-fibre diet.

Body mass and vital organ size changes

Although digestible energy intake can be maintained by an IPR, these animals would encounter negative energy balance and would have to catabolize internal energy stores during the early days of a diet shift because phenotypic responses of the GI tract are relatively slow, involving biosynthesis of new enzymes or cells (Piersma and Lindström 1997; Starck 1999a, b; McWilliams and Karasov 2001; Piersma and Drent 2003). The time required to regulate the gut capacities to match the diet shift is about 6 days in quail (Starck 1999a, b; Starck and Rahmaan 2003) and also in Brand's voles (Q.S Liu and D.H Wang, unpublished data). In this study, the 17% lighter carcass mass in Mongolian gerbils eating the diluted diet for 2 weeks implied that they fuelled the flexible responses of their gastrointestinal tract by mobilizing lipid stores from adipose tissue. Moreover, phenotypic changes in vital organs and the GI tract suggested that Mongolian gerbils allocated energy preferentially to organs used to digest, absorb and transform nutrients, or to excrete metabolites in response to the diluted diet. Downsizing of vital organs, such as the heart and spleen, suggested significant costs of gut processes (Starck 1999a, b). In addition, lower up-regulation in organ dry mass than in wet mass suggested that animals up-regulated physiological functions mainly by hypertrophy rather than hyperplasia, which probably may be advantage to save time and/or energy. In conclusion, Mongolian gerbils maintained their energy balance on the diluted diet by two strategies. An IPR maintained digestible energy intake and the energy allocation to various organs were allocated for reducing energy expenditure and up-regulating the capacity for processing food.

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